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14. ABSTRACT

This is an annual report in no cost extension period. In this period we have identified several compounds via virtual screening. These compounds include small molecules – transition state analogues and benzimidazoles. Since there is a commonality in the active site architecture, we have developed a strategy to identify inhibitors that will act on more than one serotype. Most importantly, we have determined the structure of botulinum neurotoxin type E which shows a different domain organization than either BoNT/A or B.

15. SUBJECT TERMS
Clostridium, botulinum, neurotoxin, zinc chelators, inhibitors, macromolecular crystallography, 3D structure

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Annual Report for the Period ending January 2009

Introduction

The overall major goal of this project is to design small molecule and peptidic inhibitors for botulinum neurotoxins. Botulinum neurotoxins act via a four step process: 1. Binding to neuronal cell; 2. Internalization into vesicles; 3. Translocation through endosomal membrane into cytoplasm, and 4. catalytic activity exerted on one of the three proteins forming SNARE complex required for docking and fusion to target cells for neurotransmitter release. The three structural domains are responsible for these steps and blocking any one of the steps will provide a counter measure to thwart toxicity. In our proposal we identified two targets – binding domain and catalytic domain. Our major effort is to design small molecules or peptides capable of blocking the binding of gangliosides to the binding domain or blocking the active site of catalytic domain to stop the catalytic activity. The conventional drug design is based on identifying a lead molecule and then determining the structures of lead molecules in complex with the toxin or the relevant target and then modifying the inhibitor chemically for better inhibition in an iterative manner. A two-pronged approach is being used with regard to catalytic domain. We use virtual screening of small molecule libraries to identify potential lead molecules. In the second approach, we use the substrate information to design structure and substrate based inhibitors. The general approach is to study the crystal structure of the toxin in complex with a potential inhibitor via x-ray crystallography and then analyze the interactions between the inhibitor and the protein.

Body

(1) Studies with C. neurotoxin catalytic domains

Botulinum neurotoxins are generally considered to be one of the most potent existing toxins. The neural disease, botulism is generated as a direct result of the zinc-

metalloprotease activity of each of the toxin's seven serotypes, BoNTs A through G. It is not evident upon toxin exposure as to the precise identity of the serotype involved, and thus the discovery of a broad-spectrum drug that adequately inhibits multiple BoNT serotypes would be beneficial. Previous results have depicted an overall similarity in the active-site region of multiple BoNTs that allows this enzyme to serve as a reasonable target for broad-spectrum inhibitors. In support of this outcome, we docked the potent thermolysin inhibitor, phosphoramidon to multiple serotypes and observed a similar ligand-binding mode in each serotype that resembles its conformation in the thermolysin-bound structure. We subsequently docked > 8900 bemzimdazole molecules to BoNTs A, B, C, E, F, and G, and identified thirty eight compounds that exhibited favorable energetic scores and low conformational deviations between serotypes. Eleven accessible compounds from this set were purchased and we ran inhibitory assays using BoNT A, B, C, and E. We identified four compounds that significantly inhibited at least three of the four serotypes at compound concentrations of 500 μM, despite lacking a high inhibitory potency and specificity to any single serotype. From the docked poses of these four compounds, consensus ligand-contacting residues within the BoNT binding site were determined that will be useful in guiding subsequent broad-spectrum BoNT inhibitor-design studies. A manuscript describing these results has been submitted to Journal of Computer-aided molecular design (JACMD).

(2) Crystal structure of Clostridium botulinum neurotoxin type E:

Abstract of the paper published in JMB.

Clostridium botulinum produces seven antigenically distinct neurotoxins (BoNTs A-G) sharing significant sequence homology. Based on sequence and functional similarity, it was believed their three dimensional structures will also be similar. Indeed, the crystal

structures of BoNT A and B exhibit similar fold and domain association where the translocation domain is flanked on either side by the binding and catalytic domains. Here, we report the crystal structure of BoNT E holotoxin and show that the domain association is different and unique though the individual domains are similar to BoNT A and B. In BoNT E both the binding and catalytic domains are on the same side of the translocation domain and all three have mutual interfaces. This unique association may have an effect on the rate of translocation with the molecule strategically positioned in the vesicle for quick entry into cytosol. The disease botulism caused by BoNT E sets in faster than any other serotype because of its speedy internalization and translocation and the present structure offers a credible explanation. We propose that the translocation domain in other BoNTs follows a two-step process to attain translocation competent conformation as in BoNT E. We also suggest that this translocation competent conformation in BoNT E is a probable reason for its faster toxic rate compared to A. However, this needs further experimental elucidation.

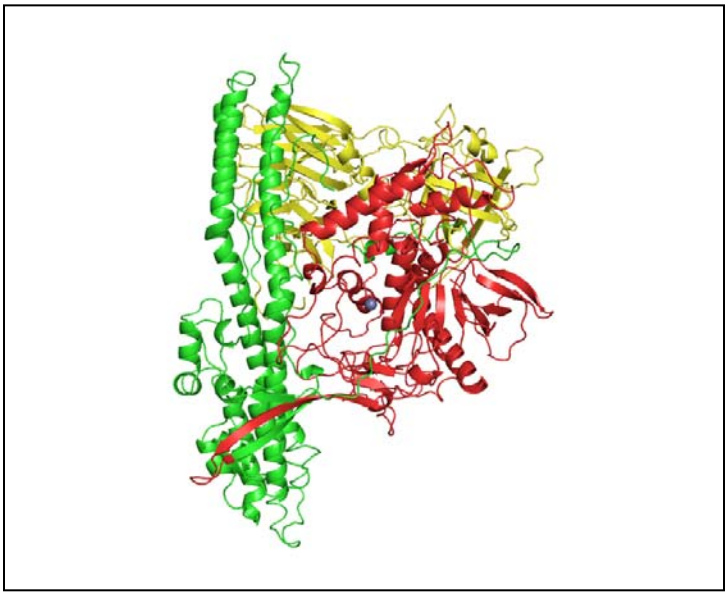

Figure 1. Ribbons representation of BoNT/E molecule.

Key Research Accomplishments

- Crystal structure of BoNT/E has been determined helping us to understand the faster action of BoNT/E compared to BoNT/A.
- A subset of benzimidazole based molecules that may inhibit multiple serotypes of botulinum neurotoxin has been identified.

Reportable outcomes

A paper on BoNT/E structure was published in JMB>

1. Kumaran, D., Eswaramoorthy, S., Furey, W., Navaza, J., Sax, M., and Swaminathan, S. Domain organization in Clostridium botulinum neurotoxin type E is unique: Its implication in faster translocation. J. Mol. Biol., 386, 233-245 (2009).

Conclusions

In our studies we have shown that it is possible to identify compounds which may inhibit multiple serotypes of botulinum neurotoxins, if not all. Also, the crystal structure of BoNT/E has shown that in spite of significant sequence homology the domain organizations of BoNT/E and A are different. Based on this we could explain the faster action of BoNT/E.

Plans for the next year:

We will complete the virtual screening of BoNT/E and B with transition state analogs.

Personnel in the Project

1. S. Swaminathan (PI)	Scientist	20% effort
2. Mike Silberstein	Research Associate	75% effort